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EXPERIMENTAL STUDIES ON THE
ORGANIZATION OF MONOAMINE NEURONS
IN THE CENTRAL NERVOUS SYSTEM

BY
ANDERS NOBIN

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From the Institute of Anatomy and Histology,
University of Lund, Lund, Sweden

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ANDERS NORLIN



The present thesis is based on the following papers:

- I. Topography of monoamine neuron systems in the human brain as revealed in fetuses. Acta physiol. scand. Suppl. 388 (1973) 1-40 (together with A. Björklund).
- II. Fluorescence histochemical and microspectrofluorometric mapping of dopamine and noradrenaline cell groups in the rat diencephalon. Brain Res. 51 (1973) 193-205 (together with A. Björklund).
- III. The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron in the rat brain. Brain Res. 51 (1973) 171-191 (together with A. Björklund, R.Y. Moore and U. Stenevi).
- IV. Organization and ultrastructural identification of the catecholamine nerve terminals in the neural lobe and pars intermedia of the rat pituitary. Z. Zellforsch. 126 (1972) 483-517 (together with H.G. Baumgarten, A. Björklund and A.F. Holstein).
- V. The adrenergic innervation of the thalamus as revealed by the glyoxylic acid fluorescence method. Comm. Dept. Anat. (Lund) 4 (1973) 1-25 (together with A. Björklund, O. Lindvall and U. Stenevi).
- VI. Effects of 5,6-dihydroxytryptamine on nerve terminal serotonin and serotonin uptake in the rat brain. Brain Res. 53 (1973) 117-127 (together with A. Björklund and U. Stenevi).
- VII. Axonal degeneration and regeneration of the bulbo-spinal indolamine neurons after 5,6-dihydroxytryptamine treatment. Brain Res. 56 (1973) 1-24 (together with H.G. Baumgarten, A. Björklund, L. Lachenmayer and U. Stenevi).

- VIII. The use of neurotoxic dihydroxytryptamines as tools for morphological studies on central indolamine neurons. Comm. Dept. Anat. (Lund) 3 (1973) 1-20 (together with A. Björklund and U. Stenevi).

References to these papers will be made by citation of the appropriate Roman numerals.

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1 INTRODUCTION

The histochemical fluorescence method developed by Falck, Hillarp and co-workers has permitted the fundamental demonstration of the cellular localization of monoamines in the central nervous system and thereby giving the necessary final evidence for the concept of monoamine-containing neurons in the mammalian central nervous system. The principal organization of noradrenaline (NA)-dopamine (DA)- and serotonin (5-HT)-containing neurons is known from many recent histochemical and chemical studies (for reviews see Fuxe, Hökfelt and Ungerstedt 1968, 1969, Moore 1970) and can be summarized as follows: The DA neurons seem to be restricted to three major neuronal systems, namely the tubero-infundibular neuron system, the nigro-neostriatal neuron system and the meso-limbic neuron system. An additional DA system has also been discovered in the retina (Malmfors 1963, Häggendal and Malmfors 1965, Ehinger 1966). The central NA neurons are not as readily classified into various distinct systems as is the case with the DA neurons. The NA cell bodies are mainly localized in medulla oblongata and pons (Dahlström and Fuxe 1964, Fuxe and Olson 1972) and many of the NA neurons seem to innervate large parts of the brain and spinal cord by way of long ascending and descending axons. These axons probably give rise to collaterals throughout the lower brain stem (Fuxe and Olson 1972). Finally, the 5-HT neuron systems originate from cell bodies in the brain stem raphe nuclei (Dahlström and Fuxe 1964). The descending 5-HT axons seem to originate mainly in the raphe nuclei of pons and medulla oblongata whereas the ascending 5-HT axons probably originate in the mesencephalic raphe region. The 5-HT axons project, similar to the NA axons, to widespread areas in the brain and the spinal cord. In addition Björklund, Falck and Stenevi (1970, 1971 a, b) have proposed the existence of a monoamine neuron system containing an

indolamine not identical with 5-HT. This system was found to consist of both ascending and descending axons originating in the raphe nuclei. Hypothetically this new proposed transmitter substance could be identical with 5-methoxytryptamine, an indolamine recently found in significant amounts in rat hypothalamus by Green, Koslow and Costa (1973).

The continuously growing morphological knowledge on the organization of the monoamine neuron systems in the central nervous system has created the necessary basis for studies on the functional importance of these amines. A wealth of data have accumulated indicating extremely important roles of central monoamines in autonomic control, neuroendocrine regulation, maintenance of sleep patterns, control of extrapyramidal motor function, elaboration of affective behavior, and learning. We are thus facing neuronal systems where morphological and functional knowledge has to a large extent developed parallelly, which has rendered possible the very fast development in the amine field.

To create the necessary detailed morphological knowledge for further continued and more sophisticated functional experiments, many new experimental techniques can be expected to be developed and adopted for neuroanatomical studies, to make possible to investigate individual problems from as many directions as possible. It seems likely that we will learn from such studies that the current knowledge on the organization of central monoamine neurons is incomplete, and that it is insufficient for the explanation of the often very intricate functional effects induced by e.g. pharmacological agents interfering with the metabolism or function of monoamine neurons.

In the studies presented in this dissertation, we have applied a variety of techniques on certain problems concerning the detailed organization of noradrenaline, dopamine and 5-HT neurons in the central nervous system of rat and man.

2 MATERIAL

As experimental animal adult albino rats of the Sprague-Dawley and Wistar strains were used. When used for experimental procedures the rats were usually 10-15 weeks old and weighed 150-300 g.

In the study on monoamine neurons in the human fetal brain (Paper I), the material comprised 35 fetuses with a crown-rump length (CRL) varying from 10 to 20 cm (gestation age about 3-4 1/2 months). The fetuses were removed by caesarean section in connexion with legal abortion. The postmortem interval preceding freezing was usually 15-30 min and in no case it exceeded 1 h. Drugs: 5-Hydroxydopamine-HBr, α -methyl-p-tyrosine-methyl ester, 6-hydroxydopamine-HCl, α -methyl-m-tyrosine-methyl ester-HCl (AB Hässle, through Dr. H. Corrodi); nialamide (Niamid [®], Pfizer); α -methylnoradrenaline (Corbasil [®], Hoffman-La Roche); phentolamine (Regitine [®], CIBA-Geigy); 4,5-dihydroxytryptamine-creatinine sulfate, 5,6-dihydroxytryptamine-creatinine sulfate (through Dr. H.G. Schlossberger, Munich); 5,7-dihydroxytryptamine-creatinine sulfate (through Dr. A. Manian, N.I.M.H., Bethesda).

3 METHODS

3.1 The formaldehyde fluorescence method (Papers I-VIII)

The formaldehyde fluorescence method of Falck and Hillarp for the histochemical demonstration of biogenic monoamines (Falck, Hillarp, Thieme and Torp 1962, Falck 1962, Carlsson, Falck and Hillarp 1962) was the central method in the present study; it is based on the principle that monoamines react with gaseous formaldehyde to yield strongly fluorescent compounds. The method has been thoroughly investigated regarding its chemistry, specificity and sensitivity (see Corrodi and Jonsson 1967, Björklund, Falck and Owman 1972). The first step in the reaction between the CA:s and gaseous formaldehyde (FA; chemical formula HCHO) is a Pictet-Spengler condensation with the formation of tetrahydroisoquinolines. Under the mild conditions used, the activation by the 3-OH group is required for this ring closure and obviously the catecholamines must be either primary or secondary amines. The next step is a dehydrogenation catalyzed by protein or amino acid giving finally, the fluorescent products, the 3,4-dihydroisoquinolines in their quinoidal state. 5-HT reacts readily and in the same principal manner as described above with an initial formation of 6-hydroxy-1,2,3,4-tetrahydro- β -carboline, and a subsequent dehydrogenation to the corresponding 3,4-dihydro- β -carboline. In recent experiments by Björklund, Falck, Lindvall and Svensson (1973) it has been shown that in addition to the 3,4-dihydro- β -carbolines formed the 1,2,3,4-tetrahydro- β -carboline is also converted through an acid-catalyzed reaction to a highly fluorescent 2-methyl-3,4-dihydro- β -carbolinium compound. For further methodological aspects and descriptions of the practical performance of the Falck-Hillarp method the reader is referred to Falck and Owman (1965), Corrodi and Jonsson (1967), Hamberger (1967), Björklund, Falck

and Owman (1972) and Björklund, Falck, Lindvall and Svensson (1973).

3.2 The glyoxylic acid fluorescence method (Paper V)

Glyoxylic acid (GA; chemical formula HOOC-CHO) has been shown to be a most powerful reagent for the fluorescence histochemical visualization of biogenic monoamines (Axelsson, Björklund and Lindvall 1972, Björklund, Lindvall and Svensson 1972, Axelsson, Björklund, Falck, Lindvall and Svensson 1973). Similar to the FA reaction, the fluorophore formation in the GA reaction involves two principal steps: First a Pictet-Spengler type cyclization to a weakly fluorescent compound, followed by the fluorophore forming reactions (Björklund, Lindvall and Svensson 1972). The reaction mechanisms underlying the formation of fluorophores and the chemical identity of the reaction products have been investigated in detail for the reaction between GA and tryptamine (Björklund, Lindvall and Svensson 1972). This study showed that the yield of fluorophores from tryptamine is considerably higher in the GA vapour reaction than in the FA vapour reaction of the standard Falck-Hillarp method, and that this higher efficiency of GA is due to the favourable catalysing properties of the carboxylic group of the GA molecule (Björklund, Lindvall and Svensson 1972).

The procedure for applying the GA fluorescence method to brain tissue is described by Lindvall, Björklund, Hökfelt and Ljungdahl (1973), Lindvall and Björklund (1973), and in Paper V. Only a short summary is given below: The rats were perfused via the ascending aorta with ice-cold 2% GA (Fluka AG, Buchs, Switzerland) in a Krebs-Ringer bicarbonate buffer (pH 7.0). After perfusion the brain tissue pieces were rapidly dissected out and cut with the Vibratome (Oxford Instruments, San Mateo, California, USA) in 20–50 μ thick sections (for details on the Vibratome technique see Hökfelt and Ljungdahl 1972, Lindvall, Björklund, Hökfelt and Ljungdahl 1973). The sections were immersed for 5 min in the ice-cold GA perfusion medium and then transferred to glass microscope slides. The slides were then dried first with warm air for 15 min and then for about 15 h in a vacuum dessicator containing phosphorous pentoxide. All sections were then treated

with GA for 2 min in 100° C (for technical details see Lindvall and Björklund 1973, Paper V). The sections were mounted in liquid paraffin before examined in the fluorescence microscope.

3.3 Microspectrofluorometry (Papers II, IV, VII, VIII)

Spectral analysis of amine fluorophores in models or in tissue sections were carried out with a modified Leitz microspectrofluorometer (see Björklund, Ehinger and Falck 1968, 1972). The excitation spectra were recorded between 300 and 450 nm with a quartz optical system for the exciting light. The emission spectra were produced with excitation at 405 nm; a secondary barrier filter with 50% transmittance at 430 nm was used.

All spectra were corrected for distorting instrument factors. Such factors influencing the emission spectra are e.g. the barrier filters, the transmission of optics and the monochromator and the sensitivity of the photomultiplier. Similarly the excitation spectra are influenced by varying transmission of the optics and the excitation monochromator and by varying intensity of the light source at different wavelengths. For correction of these factors our laboratory has adopted correction procedures designed by Ritzén (1967) (see also Björklund, Ehinger and Falck 1968, 1972, Björklund, Falck and Owman 1972, Björklund and Falck 1973). With these correction procedures, the spectra are expressed in terms of quanta per unit of wavelength.

The microspectrofluorometric differentiation between the noradrenaline and the dopamine fluorophores in Paper II was performed according to the method of Björklund, Ehinger and Falck (1968, 1972). After registration of excitation and emission spectra in the neutral (i.e. non-acidified) sections, treated with formaldehyde alone, the sections were exposed to a two-step treatment with HCl vapor in a closed vessel at room temperature. The first exposure was 15-30 sec and the second 45-120 sec, the excitation spectra being recorded after each exposure. After this acidification the dopamine and noradrenaline fluorophores showed characteristic changes which allowed the microspectrofluorometric differen-

tiation between these two catecholamines at the cellular level. The dopamine fluorophore is characterized by a shift of its main excitation peak from 410 to 370 nm upon acidification of the section; and this peak is unaffected by prolonged or excessive acidification. The fluorescence intensity is not notably affected. The noradrenaline fluorophore is characterized by a shift of the main excitation peak from 410 to 320 nm upon acidification. The peak in the excitation spectrum at 370 nm is often recorded after a slight acidification, but this peak will rapidly diminish upon prolonged acidification (see above). The fluorescence intensity is notably decreased after this treatment, which sometimes makes recordings of noradrenaline spectra difficult. When expressed as the 370:320 nm peak ratio (see Björklund, Ehinger and Falck 1972, Paper II), the acidified dopamine fluorophore is characterized by a ratio above 1.1, and the acidified noradrenaline fluorophore by a ratio of 0.8–0.9 or less. Under well-controlled conditions spectra giving intermediate ratio values (0.9–1.1) signify mixtures of dopamine and noradrenaline (Björklund, Ehinger and Falck 1972). Spectra giving intermediate ratio values were recorded from diencephalic cells dealt with in Paper II but these values were not considered since we have the opinion, due to the technically difficult brain tissue, it cannot be excluded that spectra of the intermediate type could also have been recorded from cell bodies storing dopamine or noradrenaline exclusively. For detailed information on the molecular changes of the fluorophores responsible for the shifts in excitation spectra the reader is referred to the excellent papers by Björklund, Ehinger and Falck (1968, 1972).

3.4 Electron microscopy (Paper IV)

In barbiturate anaesthetized rats the thoracic aorta was cannulated, the heart clamped, and a retrograde infusion with Periston (Bayer) initiated about 1 1/2 min after the opening of the chest. Following the Periston perfusion cold glutaraldehyde solution (about 10°C) was continuously infused over 10 min (6% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2). The pituitary neuro-intermediate lobe with the adherent pars distalis was dissected out and postfixed for 3 h in

in phosphate buffered (0.1 M) 10% osmiumtetroxide solution, containing 0.1 M sucrose, pH 7.2. Dehydration and embedding were performed according to Luft (1961) in Epon 812. Semi-thin and ultrathin sections were prepared on a Reichert ultramicrotome Om U2, using glass knives and diamond knives, respectively. Semithin sections were stained with toluidine blue pyronine, ultrathin sections were contrasted with lead citrate for 5 min according to Reynolds (1963). Electron micrographs were taken in a Philips EM 300.

3.5 Chemical analytical monoamine determinations (Papers I, IV, VII, VIII)

5-HT was estimated fluorometrically according to the method of Bertler (1961). NA and DA were determined according to Bertler, Carlsson, Rosengren and Waldeck (1958) as modified by Häggendal (1963). 4,5-DHT, 5,6-DHT and 5,7-DHT were passed through the analytical procedures used for the 5-HT and NA/DA assays and found not to interfere with the determinations. The recovery in the 5-HT determinations averaged 85%, in the NA determinations 80% and in the DA determinations 60%. Monoamine values given in Papers I, IV, VII and VIII were not corrected for recovery.

3.6 Uptake of tritiated monoamines by brain and spinal cord slices in vitro (Papers VI, VIII)

Thin, circular slices were prepared from different brain and spinal cord regions (cf. Hamberger 1967, Sachs and Jonsson 1972). The slices were preincubated in Krebs-Ringer bicarbonate buffer (pH 7.4, saturated with a mixture of 95% O₂ and 5% CO₂) and with 1.8 g glucose/l added in a metabolic shaker at +37° C; this was undertaken to meet the metabolic requirements important when incubating pieces of tissue in vitro (cf. McIlvain and Rodnight 1962). After 10 min (³H)5-HT or L-(³H)NA was added to a final concentration of 0.5×10^{-7} M and 10^{-7} M, respectively, and the slices were incubated for another 10 min in 2 ml buffer per 4-6 slices. Following incubation the slices were transferred to fresh ice-cold

buffer. In one set of experiments (Paper VI) chlorimipramine-HCl (CIBA-Geigy) was present in the incubation medium. Chlorimipramine is known to be a potent inhibitor of 5-HT uptake into 5-HT neurons in the rat brain (Shaskan and Snyder 1970, Lidbrink, Jonsson and Fuxe 1971).

To obtain values for uptake at 0°C , part of the slices obtained from control or experimental animals were incubated as above but the incubation temperature was kept at 0°C . Facilitated transport was taken as the difference between uptake measured at $+37^{\circ}\text{C}$ and uptake measured at 0°C . Uptake at 0°C was considered to be a measure of passive diffusion of 5-HT into the tissue; this uptake was 4-8% (unchanged (^3H)5-HT) and 7-15% (total radioactivity) of the $+37^{\circ}\text{C}$ uptake.

(^3H)5-HT uptake measurements. Total tritium uptake and (^3H)5-HT uptake, were measured in parallel. Each sample was homogenized in 200 μl 0.2 N acetic acid containing 20 μg non-radioactive 5-HT creatinine sulphate, and then centrifuged at about $3\,000 \times g$ for 10 min. 90-95% of the tissue radioactivity was recovered in the supernatant. From each supernatant 2 aliquots of 10 μl (for duplicate determinations) were directly added to scintillation vials for determination of total tritium, and two 10 μl aliquots were spotted onto silica gel thin-layer plates (Kieselgel H, Merck, Darmstadt, W. Germany) for chromatographic isolation of unchanged (^3H)5-HT. The plates were developed in ethylacetate-isopropanol-ammonia (9 : 7 : 4) with 2 mg EDTA/100 ml added. In this system 5-HT is well separated from its major metabolite 5-hydroxyindolacetic acid (5-HIAA) (cf. Axelsson, Björklund and Seiler 1971). The 5-HT spot was scraped off the plate, including a 5 mm wide area around the visible spot, the powder was mixed with 0.5 ml water in the scintillation vial, and 10 ml scintillation fluid were added. The extraction of radioactivity from the silica gel powder was virtually complete (cf. Fleming and Clark 1970). The recovery of added 5-HT in the entire procedure was well reproducible and averaged 45%. The (^3H)5-HT uptake values reported in Papers VI and VIII were corrected for recovery.

(^3H)NA uptake measurements. The incubated slices were directly dissolved in 0.5 ml Soluene 100 (Packard Instr. Co.) and then subjected to

liquid scintillation counting. The possible metabolism of NA in the incubated slices and in the incubation medium was considered. It is reported that NA dissolved in buffer solutions of various pH:s between 6-8 remained unchanged at 37° C for at least 30 min (Sachs 1970) and our results (Paper VI) showed that after extraction in 0.2 N acetic acid and chromatography on cellulose thin-layer plates unchanged NA constituted 85-100% of total radioactivity (cf. Fleming and Clark 1970).

In the scintillation counting, the counting efficiency was calculated by means of internal standards.

3.7 Stereotaxic lesions (Papers II, III, V, VII)

All surgical procedures were carried out under general anaesthesia using either halothane plus N₂O and O₂, or Brietal (Lilly, 40 mg/kg). Surgery was performed under clean but not aseptic conditions. All intracranial operations were performed with the animals placed in a Kopf stereotaxic instrument for rats. The coordinates for stereotaxically placed lesions were obtained from the atlas of König and Klippel (1963). Electrolytic lesions (Papers II, III and V) were produced using a needle-shaped, insulated stainless steel electrode with an exposed tip approximately 0.2 mm long. The electrode was placed in the brain to a previously determined set of coordinates. A neutral electrode was placed in the rectum. The lesion was produced by passing a sinusoidal wave at 1.4 mA. With this technique the duration of current passing determines lesion size. Vertical knife lesions (Papers II, III, V) were produced with a microknife which had a horizontal cutting edge 1.2 mm or 2.0 mm long. The knife was fixed into the stereotaxic instrument and a lesion was made by vertically lowering the knife through the brain substance to the desired depth. Halász-type lesions (Papers II and III) were performed with a modified Halász knife which had vertical and horizontal components each 2.4 mm long. The lesions were made according to the technique described by Halász and Pupp (1965). The knife was placed in the stereotaxic instrument and then lowered into the appropriate position through a slit in the superior sagittal sinus.

The knife was then rotated, returned to the original position and withdrawn.

Aspiration lesions (Paper III) were performed by aspiration of brain tissue through a small-bore pipette under direct vision. Spinal cord compressions (Paper VII) were performed at the mid-thoracic level. One or two laminae were cut on both sides and the spinal cord was then compressed for 20–30 sec with a pair of fine tweezers. Cervical sympathectomy (Papers III and IV) was performed by bilateral removal of the superior cervical ganglia.

3.8 Stereotaxic injections (Papers VI, VII and VIII)

Three different dihydroxytryptamines were administered stereotaxically into various areas of the brain by injection with a motor driven syringe through a cannula with an outer diameter of 0.4 mm. All drugs were injected under barbiturate anaesthesia (Brietal, Lilly 40 mg/kg). The substances were dissolved in dilute mammalian Ringer (1/3 distilled water and 2/3 Ringer) containing 0.1 mg/ml ascorbic acid or in saline. Intraventricular injections (Papers VI, VII and VIII). Ten, 25, 50 and 75 µg (free base) of 5,6-DHT (creatinine sulfate-H₂O complex), and 50 and 150 µg (free base) of 5,7-DHT (creatinine sulfate-H₂O complex) were stereotaxically injected into the lateral ventricle. The dihydroxytryptamines were all dissolved in 20 µl dilute mammalian Ringer. Intracerebral injections (Paper VIII). 4,5-DHT, 5,6-DHT and 5,7-DHT were injected into a number of sites where various 5-HT-containing cell groups, axonal pathways and terminal areas are located. Usually the injected volume was 4 µl and the concentration of drug 1 µg/µl. Intraspinal injections (Paper VIII). Two injections of 5,7-DHT, 2 µg in 2 µl each, were given in between the 2nd and 3rd cervical vertebrae. The vertebral column was fixed and stretched so that the position of the spinal cord was unaffected by the breathing of the animal. The control animals received saline similarly. The injections were done at a speed of 1 µl/min.

4 RESULTS AND COMMENTS

4.1 Topography of monoamine neurons in the human fetal brain (Paper I)

Considering the great interest in the functional significance of the monoamine neurons in the CNS of man it is remarkable how so little is known about the cellular localization of biogenic amines in the human brain. The major reason for this lack of knowledge is that this material offers special technical problems. Although the brain levels of catecholamines and serotonin decline rather slowly during the first hours after death (Bertler and Rosengren 1959, Joyce 1962, McGeer and McGeer 1962) their fluorescence histochemical visualization in central nervous tissues is possible only when the tissue is processed within the first 30-60 min after death (Björklund, Falck and Rosengren 1967, De La Torre 1972, Björklund and Nobin unpublished observations). We have circumvented this obstacle by investigating fresh brains from 3-4 months old human fetuses.

The present study (Paper I) demonstrates the localization of catecholamines and indolamines in nerve cell bodies, axons, and axon terminals in the human brain. At the developmental stage studied (gestation age 3-4 months) the CA and IA cell groups and major axon pathways were richly developed, whereas fluorescent varicose, probably terminal CA-containing fibres occurred abundantly only in certain well-developed regions such as the hypothalamus, the basal ganglia and the septal and olfactory regions. The low concentrations of NA and DA (between 10 and 20 ng/g of tissue) in the whole brains of the 3-4 month old fetuses are thus primarily due to the limited development of the terminal axon network (Paper I). In Paper I a detailed description of the distribution of monoamine neurons in the fetal brain is given and in this summary only a few points of particular interest will be dealt with.

The fluorescence microscopical demonstration of a nigro-striatal CA pathway in the human brain is of particular interest. Although there is much indirect evidence for such a system also in man, e.g., from observations in parkinsonian patients (see Hornykiewicz 1966), its actual demonstration has been lacking. Due to the high fluorescence of the monoamine-containing fibre bundles in the fetuses, the nigro-striatal system could be mapped from the substantia nigra into the internal capsule and the globus pallidus. The course of the fibres revealed in this way has clear similarities to that described by Carpenter and Peter (1972) in the monkey, and by Moore, Bhatnagar and Heller (1971) in the cat.

In the developing brain of human fetuses, CA-containing varicose fibres were demonstrated not only in the caudate nucleus and the putamen, but also in the developing globus pallidus. This conforms well to the biochemical findings of moderate to high concentrations of dopamine in these regions in adults (Bertler and Rosengren 1959, Sano, Gamo, Kakimoto, Taniguchi, Takesada and Nishinuma 1959, Ehringer and Hornykiewicz 1960, Bertler 1961, Hornykiewicz 1966, Fahn, Libsch and Cutler 1971) and in an 8 months old human fetus published by Bertler (1961). As the globus pallidus appears to be devoid of CA-containing terminals in the rat (Fuxe 1965, Fuxe, Hökfelt and Ungerstedt 1969) it has been questioned whether the dopamine present in this structure in man is confined to non-terminal fibres passing through the nucleus to the neostriatum or to actual terminals innervating the nucleus (Hornykiewicz 1966). The present finding provide evidence for a direct CA (probably mainly dopamine-containing) fibre supply to the globus pallidus, suggesting that in man the mesencephalic dopamine-containing neurons might project also to globus pallidus. Nigro-pallidal fibre connexions have been presumed to exist in the human brain (Hornykiewicz 1966) but neither in cat (Moore, Bhatnagar and Heller 1971) nor in monkey (Carpenter and Peter 1972) a substantial termination of nigral fibres has been possible to demonstrate in the globus pallidus with silver staining of degenerating terminals. Thus, until direct evidence for a nigro-pallidal projection has been obtained in man, the possibility has to be considered that the CA-fibres in the globus pallidus originate in other

areas, e.g. in mesencephalic CA cell bodies outside the substantia nigra.

The observations reported in Paper I might also be of significance for the interpretation of the findings of varying, but significant, levels of dopamine and noradrenaline in the nucleus ruber in the human brain (Sano, Gamo, Kakimoto, Taniguchi, Takesada and Nishinuma 1959, Ehringer and Hornykiewicz 1960, Bertler 1961). As in the rat, the nucleus was devoid of CA-containing structures in the human fetus, but the ascending CA bundles from medulla-pons and from mesencephalon passed close to the nucleus in a capsule-like arrangement. These ascending CA-bundles thus seem to be the likely source of the CA:s detected in preparations of the nucleus ruber.

Hypothalamus is one of the probable areas of termination of the ascending CA fibre system from the medulla and pons, being the region with the highest concentrations of noradrenaline in the brain of the adult (Bertler and Rosengren 1959, Bertler 1961, Hornykiewicz 1966, Fahn, Libsch and Cutler 1971) as well as of the 8 months old fetus (Bertler 1961). This corresponded to a rich supply of varicose CA-containing fibres in several hypothalamic nuclei in the present 3-4 months old fetuses. Apart from this extrinsic, probably noradrenergic, innervation, there occur in the rat diencephalic noradrenaline and dopamine-containing cell groups (Fuxe, Hökfelt and Ungerstedt 1969, Paper II) that most probably have intrinsic diencephalic projections (Papers III and V). Björklund and Nobin (Paper II) have distinguished four such cell groups, and of these, only cells corresponding to the tubero-hypophyseal dopamine group (group A12) were detected in the human fetuses. In the rat, these neurons have their terminals in the median eminence, the neural lobe, and the pars intermedia (Björklund, Falck, Hromek, Owman and West 1970, Jonsson, Fuxe and Hökfelt 1972, Papers III and IV) and from the present fluorescence histochemical findings, it seems probable that the organization is similar in man with respect to the innervation of the median eminence and the neural lobe. But no CA innervation of the pars intermedia cells was disclosed in the human fetal pituitaries (Björklund and Nobin unpublished observations). The demonstration of tubero-hypophyseal CA (probably dopamine)-containing neurons in the human brain is interesting in view of the important role these

neurons play in the release of hypophysiotrophic hormones from the median eminence in the rat (for reviews, see Hökfelt and Fuxe 1972, McCann, Kalra, Donoso, Bishop, Schneider, Fawcett and Krulich 1972). The morphological basis for such a function thus seems to exist also in man.

4.2 Characterization of the diencephalic CA cell groups (Paper II)

The prominent hypothalamic and thalamic DA and NA cell groups described in Paper II and, to a limited extent, also in Papers III and V have in the literature been largely neglected. In fact the possible existence of NA cell bodies in the diencephalon have been totally overlooked. We felt, however, that a detailed neuro-anatomical knowledge of these neuronal systems is desirable and should provide a basis for studies on their roles in diencephalic functions. Therefore in a combined fluorescence microscopical and microspectrofluorometric study, the distribution, morphology and amine content of diencephalic CA-containing cell groups were investigated in the rat brain (Paper II). Topographically the diencephalic CA cell bodies are confined to two fairly continuous cell systems well separated from each other: one mainly located in the periventricular region of the rostral and middle hypothalamus, and one in the dorsal and caudal hypothalamus and caudal thalamus. On the basis of morphology and amine content, 4 main diencephalic cell groups have been distinguished: (1) The rostral periventricular DA cell group; (2) the DA cell group of the arcuate nucleus, constituting the dopaminergic tubero-hypophyseal neuronal system; (3) the dorsal hypothalamic DA cell group; and (4) the caudal cell group, comprising both DA-containing and NA-containing cell bodies, extending from the periventricular grey matter of the caudal thalamus down into the posterior hypothalamic area.

Our investigation (Paper II) thus showed prominent systems of both DA-containing and, not earlier reported, NA-containing cell bodies in the rat diencephalon.

4.3 Terminal projections of the diencephalic cell groups (Papers III and V)

The knowledge of the terminal projections from the diencephalic cell groups is limited to some information on the axonal pathways from the DA cell bodies in the arcuate nucleus. The lesion experiments performed by Fuxe and Hökfelt (1966) and the noradrenaline uptake studies performed by Lichtensteiger and Langemann (1966) suggested that the CA-storing cell bodies found in the arcuate nuclei have their terminals in the pituitary, probably in the median eminence. Pharmacological experiments with α -methyl-m-tyrosine and desimipramine (Carlsson, Falck and Hillarp 1962, Fuxe 1964, Fuxe, Hamberger and Malmfors 1966) indicated the presence of DA in the median eminence. The study of Björklund, Falck, Hromek, Owman and West (1970) demonstrated two prominent neuron systems in the hypothalamo-hypophyseal complex: one containing DA and probably originating from the cells in the arcuate nuclei and one containing NA. Moreover, that study suggested the involvement of a third neuron system possibly containing an indole derivative (cf. also Björklund, Falck and Stenevi 1970, 1971 a, b). Our investigation (Paper III) aimed at elucidating the contribution of diencephalic as well as mesencephalic and pontine cell groups to the innervation of the very important median eminence-pituitary region. One obstacle which had to be overcome before performing the study was to get a safe and reproducible picture of the innervation pattern in the median eminence and in the neuro-intermediate lobe. By using the special precautions in the histochemical procedure introduced by Björklund and Falck (1968) and by using systemically administered α -m-NA these difficulties were eliminated. α -m-NA is selectively taken up by central and peripheral adrenergic neurons (Hökfelt 1968, Iversen 1970) and this mechanism probably accounted for the consistent fluorescence picture obtained in the present study.

Apart from the vascular, sympathetic innervation only one system of pituitary CA fibres was established to originate outside the mediobasal hypothalamus, namely part of the innervation of the internal and subependymal layers of the median eminence. In agreement with the findings of Ungerstedt (1971) these

fibres were removed by bilateral lesions of the ascending CA fiber system in the caudal mesencephalon, a fiber system that is probably comprised of NA axons (Ungerstedt 1971, Fuxe and Olson 1972). In Paper III this system is named the reticulo-infundibular NA system. The remaining components of the median eminence-pituitary CA innervation were removed only by lesions in the arcuate and periventricular nuclei of the mediobasal hypothalamus. In principle, the denervation effects of these lesions could be due either to the destruction of the cell bodies of origin, or to section of fibres of passage through the area of the lesion. However, since more distant lesions deafferenting the mediobasal hypothalamus did not affect the remaining CA innervation, it seems highly probable that the effective lesions denervated the median eminence-pituitary region by destroying cell bodies giving rise to its innervation. The results presented in Paper III show that the bulk of terminals in the median eminence-pituitary proper region originates within the DA neurons situated in the arcuate nuclei and the ventral parts of the adjacent periventricular nuclei (areas A12 and A14 according to Dahlström and Fuxe 1964 and Paper II). This tubero-hypophyseal neuron system can be separated into 3 different components (Paper III). One is an arcuato-infundibular system terminating in the external and, most probably, in the internal and subependymal layers of the median eminence and the stalk. A second system originates in the most rostral 0.2-0.3 mm of the arcuate nuclei and innervates the whole pars intermedia. The third system, innervating the neural lobe proper, originates in a small well-defined area of the arcuate nuclei situated immediately caudal to the area of origin for the pars intermedia innervation.

The projections from the fluorescent cell bodies in the arcuate nuclei and in the ventral parts of the periventricular hypothalamic nuclei thus seem to project in an intricate and complex manner to the median eminence-pituitary region. This gives a morphological basis for the interpretation of the probably complex role of the CA:s in the neuroendocrine regulations in the median eminence and in the neurointermediate lobe.

No suggestions as to terminal projections from the dorsal hypothalamic

cell group and from the caudal cell group (for nomenclature, see Paper II) are given in the literature. In fact, it seemed possible that the Falck-Hillarp technique is not sufficiently sensitive to reveal terminal areas that could be considered as projections from the above-mentioned cell groups. When the glyoxylic acid fluorescence method was introduced to neuroanatomical research (Lindvall and Björklund 1973, Paper V), new possibilities for detailed anatomical studies opened up owing to the higher sensitivity and precision of this method. Thus, in a combined glyoxylic acid fluorescence and stereotaxic study (Paper V), we could disclose an earlier unreported system of fluorescent terminals in the diencephalon of the rat. This extensive terminal system extended from the subparafascicular nucleus and rostrally through the zona incerta and finally extended ventrally into the anterior hypothalamic nucleus. The dorsal hypothalamic cell group and part of the caudal cell group were closely associated with this terminal system, suggesting that the fibres originated in these cell groups. In fact, the terminal system was not affected by large lesions in the substantia nigra or in the dorsal catecholamine bundle. These observations can be taken as evidence for the existence of intradiencephalic CA neuron systems, in addition to the previously known tubero-infundibular DA system (Paper V). Though no direct observations of the organization of these intradiencephalic CA systems were possible it seems justified to presume that the DA cell bodies in the dorsal hypothalamic cell group and in the caudal cell group are the source for the CA terminals in the zona incerta and in the anterior hypothalamic nucleus.

The lesion experiments did not provide any definite information concerning the projections of the diencephalic NA neurons. From the glyoxylic acid fluorescence picture, it seems probable, however, that the NA cell bodies belonging to the caudal cell group (see Paper II) contribute to part of the NA innervation of certain thalamic nuclei (Paper V).

4.4 Fine structural relations between CA-fibres and hormone-producing structures in the pituitary neuro-intermediate lobe (Paper IV)

Fluorescence histochemical studies have demonstrated that the mammalian neuro-

-intermediate lobe receive abundant catecholamine innervation of central origin (Dahlström and Fuxe 1966, Björklund, Falck and Rosengren 1967, Björklund 1968, Björklund, Enemar and Falck 1968, Björklund and Falck 1969, Weman and Nobin 1973, Paper III). The electron microscopical identification of catecholamine terminals in the mammalian central nervous system presents special problems, however, (see Hökfelt 1968) and although the mammalian neuro-intermediate lobe has been subjected to several electron microscopical investigations (for refs. see Paper IV) the catecholamine terminals have not been unequivocally identified. The problem of the ultrastructural identification of catecholamine fibres in the mammalian neuro-intermediate lobe has been considered in the studies by Bargmann, Lindner and Andres (1967), Cannata and Tramezzani (1969), Vincent and Anand Kumar (1969) and Belenky, Konstantinova and Polenov (1970). These authors described a fibre type equipped with small to large dense-cored vesicles (ranging from 500 to 1 400 Å in diameter) which they tentatively identified as adrenergic.

In the present investigation (Paper IV) we used the dopamine analogous 5-hydroxydopamine (5-OH-DA) and 6-hydroxydopamine (6-OH-DA) in a combined fluorescence histochemical, microspectrofluorometric and electron microscopical study to identify ultrastructurally the central catecholamine innervation in the rat neural lobe and pars intermedia, and to obtain more detailed information on their fine structural arrangement.

The fibre type that was identified as catecholamine-containing was ultrastructurally chiefly characterized by dense-cored vesicles, 500- 1 200 Å in diameter, intermingled with varying numbers of small empty vesicles. 5-Hydroxydopamine was selectively accumulated in these fibres and caused an increased electron density of the granular vesicles as well as of some small normally agranular vesicles, and systemically administered 6-hydroxydopamine caused a selective degeneration of these fibres, most prominently within the neural lobe. The dopaminergic terminals of the neural lobe showed frequent close contacts (80-120 Å), without real membrane thickenings, to neurosecretory axons and to pituicyte processes. It is suggested that these close contacts might signify a direct dopaminergic influence on the neuro-secretory axons and/or on the pituicytes. The

identified central catecholamine fibres were also found to make common synapse-like contacts on the pars intermedia cells, whereas the innervation by neurosecretory fibres was very rare in the pars intermedia. This suggests that the direct central nervous control of the rat pars intermedia is exerted by the catecholamine neurons. A very special feature of the catecholamine fibres in the pituitary is the occurrence of peculiar, large dopamine-filled droplet-like swellings. Electron microscopically, such large axonal swellings (more than $2\ \mu$ in diameter) were found to contain, in addition to the characteristic vesicles and organelles, strongly osmiophilic lamellated membrane complexes resembling myelin bodies and multivesicular bodies encircling disintegrated vesicles, suggesting that these "droplet fibres" represent dilated stumps of spontaneously degenerating dopaminergic axons. It is suggested that the dopaminergic neural lobe fibres are undergoing continuous reorganization through degeneration-regeneration cycles, a phenomenon previously suggested for the neurosecretory axons of the neural lobe (Paper IV).

4.5 The adrenergic innervation of the thalamus (Paper V)

Fuxe (1965) reported in a formaldehyde fluorescence microscopical investigation that most nuclei in the thalamus had a very low to low density of NA terminals. The only thalamic nuclei with a significant NA innervation were the anterior thalamic nucleus and part of the paraventricular thalamic nucleus (Fuxe 1965). It has been proposed that the NA terminals in the thalamus originate in the NA cell bodies within the locus coeruleus. The axons reach the thalamus via the dorsal catecholamine bundle (Ungerstedt 1971, Olson and Fuxe 1972, Maeda and Shimizu 1972). In view of the important role that different parts of the thalamus play for the understanding of motor disorders, including Parkinson's disease and the involvement of monoamines in the latter disease, a deeper knowledge of the distribution of adrenergic fibres in the thalamus seemed highly desirable.

The very high sensitivity of the glyoxylic acid fluorescence method enabled us to perform a detailed study on the origin and distribution of the thalamic catecholamine innervation (Paper V). This method not only permits the

visualization of catecholamine terminals, but also of the entire preterminal axons; it has therefore made possible an exact description of the different axonal pathways leading to the thalamus region (Lindvall and Björklund 1973, Paper V).

The normal innervation pattern in the different nuclei of the thalamus proved much richer than earlier reported. Very interesting were the observations that areas regarded as devoid or almost devoid of CA innervation actually contained a highly significant number of fluorescent terminals and fluorescent preterminal fibres. A good example of this is the discovery of a very dense terminal area in the subthalamus, which extends also into the zona incerta and the anterior hypothalamic area. Paper V gives a detailed mapping of the thalamic CA innervation, as revealed with the glyoxylic acid fluorescence method.

The origin of most of the thalamic fluorescent fibres proved to be NA cell bodies in the lower brain stem (Paper V; cf. Ungerstedt 1971 a, Olson and Fuxe 1972, Maeda and Shimizu 1972). Cutting the ascending NA pathways in the caudal mesencephalon achieved a practically complete denervation in most thalamic nuclei, except in the anteroventral nucleus and the periventricular nucleus, which were only partially reduced. It seems possible that remaining fibres could be intrinsic thalamic projections from the thalamic NA neurons (see above; cf. Paper II). For further discussion on the origin of the thalamic CA innervations see Paper V.

4.6 Effects of dihydroxytryptamines on central monoamine neurons (Papers VI, VII and VIII)

When the remarkable, acute, and selective degenerative effect of 6-hydroxydopamine (6-OH-DA) on peripheral sympathetic nerve terminals was demonstrated (Tranzer and Thoenen 1967, 1968, Thoenen and Tranzer 1968, Malmfors and Sachs 1968), the concept of selective chemical denervation was introduced in neurobiological research. Accumulated findings provide strong evidence for a cytotoxic effect of 6-OH-DA also on brain CA neurons (Ungerstedt 1968, 1971 b, Bloom, Algeri, Groppetti, Revuelta and Costa 1969, Uretsky and Iversen

1970).

In such a complex and morphologically intricate structure as the brain, the selective chemical denervation is a most attractive approach to neuroanatomical and functional problems, particularly when dealing with such widespread and diffusely organized systems as the monoamine neuron systems. It is obvious that chemical denervation offers possibilities for overcoming the drawbacks inherent in denervation through mechanical or electrolytic lesions, which in brain inevitably involves a number of different neuron systems and non-neuronal tissue elements. Considering the potential usefulness of selective neurotoxic pharmacological agents for neuroanatomical and functional studies, it was felt of uttermost interest to develop drugs with such properties for indolamine neurons in the CNS.

In 1971 Baumgarten, Björklund, Lachenmayer, Nobin and Stenevi introduced the serotonin analogue 5,6-dihydroxytryptamine as a tool for the induction of a selective destruction of serotonin containing neurons in the brain of mammals. Subsequent extensive chemical, histochemical and electron microscopical work (Baumgarten, Björklund, Lachenmayer, Nobin and Stenevi 1971, Baumgarten, Evetts, Holman, Iversen, Vogt and Wilson 1972, Baumgarten, Victor and Lovenberg 1973, Baumgarten, Lachenmayer and Schlossberger 1972, Baumgarten, Björklund, Holstein and Nobin 1972, Daly, Jonsson and Fuxe 1973, Papers VI, VII and VIII) established that 5,6-DHT acts on indolamine neurons in principally the same manner as 6-OH-DA on CA-containing ones.

In the present investigations (Papers VI, VII and VIII) the detailed effects of 5,6-DHT and, to a minor extent, also the effects of 4,5-DHT and 5,7-DHT on the serotonin and catecholamine neurons in the central nervous system were studied (for information on 4,5-DHT and 5,7-DHT, see also Baumgarten and Lachenmayer 1972, Baumgarten, Björklund, Lachenmayer and Nobin 1973 a, b).

One intraventricular injection of 5,6-dihydroxytryptamine (5,6-DHT) caused the disappearance of fluorescence histochemically detectable 5-HT terminals and a loss in 5-HT uptake sites (Paper VI). There was an almost complete disappearance of 5-HT-containing nerve terminals in periventricularly located diencephalic areas and in the spinal cord 10-15 days after 75 µg of 5,6-DHT.

The noradrenaline and dopamine innervation patterns in the hypothalamus, septum, basal ganglia, and spinal cord appeared normal except in a narrow zone of the caudate nuclei facing the lateral ventricles, where there was a marked reduction in dopamine fluorescence. These changes were accompanied by 50-87% reductions in the uptake of (^3H)5-HT by thin slices of cortex, hypothalamus and spinal cord in vitro. In contrast, the uptake of L-(^3H)NA was close to normal in hypothalamus and spinal cord slices, and about 35% reduced in the cortex slices. These results indicate that intraventricularly administered 5,6-DHT causes extensive axonal degeneration of central serotonin neurons, and that noradrenaline and dopamine neurons are largely unaffected after one injection of 75 μg . To further investigate the cellular events induced by 5,6-DHT in 5-HT-neurons, a well-defined 5-HT neuronal system, namely the bulbo-spinal system, was selected and subjected to an extensive study (Paper VII). The results indicated that, in the bulbo-spinal system, 5,6-DHT has a direct neurotoxic action primarily on the superficially located non-terminal parts of the indolamine-containing axons, and that terminal degeneration largely occurs as an anterograde degeneration of the lesioned axons. The indolamine cell bodies were not acutely damaged but some of them disappeared 1-3 months after injection, probably as a result of retrograde degeneration.

4.7 Dihydroxytryptamines as tools for morphological studies on central indolamine neurons (Paper VIII)

Morphological studies of indolamine neurons offer special problems due to insufficient sensitivity of available fluorescence histochemical methods. For this reason the knowledge of the course of the axonal pathways and the distribution of their terminals is still very incomplete. Based primarily on studies with mechanical or electrolytic brain lesions, a major ascending serotonin-containing fibre system has been described, that originates in cell groups of the mesencephalic and pontine reticular formation (Dahlström and Fuxe 1964) and reach via the medial forebrain bundle to innervate different forebrain structures (Heller, Harvey

and Moore 1962, Andén, Dahlström, Fuxe, Larsson, Olson and Ungerstedt 1966, Moore and Heller 1967, Fuxe, Hökfelt and Ungerstedt 1968, Moore 1970, Ungerstedt 1971 a). But details on the topography of the several components of this system are still lacking (see Moore 1970), at least partly owing to the difficulties in visualizing and tracing the indolamine fibres with the mechanical or electrolytic lesioning techniques.

In Paper VIII the usefulness of the recently introduced neurotoxic serotonin congeners, 5,6-DHT, 5,7-DHT and 4,5-DHT, for morphological studies and localized denervations of central indolamine neurons have been investigated in the rat brain.

Both intraventricular and intracerebral injections of 5,6-DHT or 5,7-DHT proved very efficient for the visualization of non-terminal indolamine fibre bundles from their cells of origin for long distances through the main fibre bundles. In this way the ascending MFB system of indolamine fibres became clearly visible and during its course through hypothalamus and the septal region the branching of the fibres from the MFB system could be observed. Of special interest is that apart from the previously established ventral bundle of indolamine fibres ascending from the mesencephalon a dorsal ascending indolamine fibre bundle was revealed. This bundle appeared to originate in the pontine raphe cell groups, and possibly also in the dorsal raphe nucleus in the mesencephalon, and ran in the medial longitudinal fasciculus up to the meso-diencephalic junction where it turned ventrally to join the ascending MFB system. Interestingly, the course of this dorsal indolamine bundle is fairly parallel to the so-called dorsal CA bundle (Ungerstedt 1971).

A second finding of particular anatomical interest is the failure of injections of dihydroxytryptamines in the ventromedial tegmentum to remove the indolamine fibres of the pretectal area, although these injections resulted in a complete or almost complete disappearance of IA fibres in other forebrain regions where it was possible to visualize indolamine nerve fibres, reflected in a 90-94% reduction in the (^3H)5-HT uptake in hypothalamus and cortex. Thus,

it seems appropriate to postulate a different source of origin of the remaining fibres in the pretectal region. Hypothetically, this source could be the serotonin-containing cells described by Björklund, Owman and West (1972) in the medial part of the medial habenular nucleus and in the so-called lamina intercalaris, situated at the base of the pineal stalk in between the posterior and the habenular commissures. Such a short epithalamic serotonin neuron system would constitute a link between the habenula—and possibly also the pineal—and the pretectal region.

The efficiency of intracerebral injections of 4,5-DHT, 5,6-DHT and 5,7-DHT for regional denervations in the CNS was tested in two locations: (1) after injection in the ventromedial tegmentum close to the ascending IA fibre system, and into the grey matter of the cervical spinal cord close to the descending bulbo-spinal IA fibre system. According to the combined fluorescence microscopical and biochemical results the injection of 4 μ l and 2 μ l respectively of the dihydroxytryptamines (1 μ g/ μ l) into these sites produced a very efficient destruction of the IA fibre tracts. 5,7-DHT, which was the most efficient compound, thus caused a 85% reduction in forebrain 5-HT concentration and a 90-94% reduction in (3 H)5-HT uptake in slices from hypothalamus and cortex 10 days after its injection into the ventromedial tegmentum. With surgical lesions reductions of this magnitude are obtained only after large lesions destroying almost the entire midbrain raphe region (Kuhar, Roth and Aghajanian 1971, Kuhar, Aghajanian and Roth 1972). The efficiency of the 5,7-DHT injections is also illustrated by the spinal cord experiments, where the effect of 5,7-DHT on the IA neurons was comparable to that obtained after a total transection of the cord.

Concerning the effects of 4,5-DHT, 5,6-DHT and 5,7-DHT on catecholamine neurons it is obvious that the degenerative action of the dihydroxytryptamines is selective for serotonin neurons only within a certain dose range. With the dose used for the intracerebral injections in Paper VIII all three compounds probably caused a partial damage to the ascending dopamine systems. The effect on the noradrenaline neurons, on the other hand, was different between 5,7-DHT that

caused a significant damage to NA fibres and 4,5-DHT and 5,6-DHT that left these neurons practically intact. This difference in the sensitivity of the NA neurons to the different dihydroxytryptamines conforms well to the observations made after intraventricular injections (Baumgarten, Björklund, Lachenmayer, Nobin and Stenevi 1971, Baumgarten, Björklund, Lachenmayer and Nobin 1973, Baumgarten, Lachenmayer, Björklund, Nobin and Rosengren 1973, Paper VI) and is probably explainable by the different affinities for the catecholamine membrane pump by the different dihydroxytryptamines. Thus, one can expect a preferential affinity of the 7-OH group in 5,7-DHT to the uptake systems of CA neurons, since 7-hydroxytryptamine (7-HT) itself mainly interferes with the amine uptake mechanisms of CA neurons (Horn and Baumgarten, personal communication). On the contrary, 6-hydroxytryptamine (6-HT) competes quite well for uptake into serotonin neurons and the 6-OH group does not notably interfere with the uptake of 5,6-DHT into 5-HT neurons. Thus, 5,6-DHT exerts more selective effects on central serotonin neurons than 5,7-DHT, but is limited in its applicability for selective degeneration in brain because of the higher inherent general toxicity and chemical lability.

As pointed out in Papers VI, VII and VIII, when using the dihydroxytryptamines for morphological or functional studies, consideration must always be paid to the general tissue damage caused by the drug. By all probability these compounds are generally cytotoxic and selective damage is achieved only when the general tissue concentration of the drug is so low that toxic levels are reached only within those tissue elements (i.e. the monoamine neurons) that actively concentrate the drug by means of the so-called membrane pump. This holds true also for the effects caused by 6-OH-DA. Poirier, Langelier, Roberge, Boucher and Kitsikis (1972) have clearly shown that, when given in too high amounts or in too high concentrations, 6-OH-DA can produce almost unlimited tissue damage. Thus, as in the case of surgical brain lesions, the nature, localization and extent of the chemical lesions have always to be checked microscopically; biochemical controls alone can obviously be entirely mis-

leading. Since the neurotoxic amines have no absolute specificity for monoamine neurons it seems likely that extensive damage of the monoamine neuron systems is always accompanied by a certain amount of damage also to other brain elements that are exposed to high concentration of the drug (cf. Baumgarten, Björklund, Holstein and Nobin 1972). This might be of importance particularly when the neurotoxic amines are used for functional studies.

4.8 Regeneration of indolamine neurons after chemical lesioning (Paper VII)

Catecholamine and indolamine neurons in the adult rat brain have been found to possess remarkable growth capacity after axonal damage (Katzman, Björklund, Owman, Stenevi and West 1971, Björklund and Stenevi 1971, Björklund, Katzman, Stenevi and West 1971, Moore, Björklund and Stenevi 1971). By monoamine histochemistry this is observed after mechanical or electrolytic lesions as vigorous sprouting from the severed axons.

In the present investigation (Paper VII) the appearance of new delicate, indolamine-containing fibres, probably identical with new axonal sprouts could be observed 10 days and onwards after 5,6-DHT treatment. Three months after injection these newly appearing fibres occurred abundantly in large areas of the medulla oblongata and in the most cranial part of the spinal cord. These fluorescence histochemical observations parallel an increase in uptake of (^3H)5-HT in slices from medulla oblongata and the cranial spinal cord 3 months after 5,6-DHT treatment as compared to the uptake 10 days after injection (Björklund, Nobin and Stenevi 1973). The results are also in line with the data published by Baumgarten, Lachenmayer, Björklund, Nobin and Rosengren (1973) showing a gradual chemical recovery of 5-HT concentrations in all brain regions to normal or even supra-normal values 2-4 months after injection of 75 μg 5,6-DHT. The recovery in the spinal cord, however, was not significant within 6 months after injection. We are thus facing the possibility of regenerative sprouting in brain of chemically lesioned indolamine axons that eventually may result in a partial anatomical and functional reinnervation of brain centres previously deprived of their terminal indolamine innervation.

5 CONCLUSIONS

A. In a fluorescence histochemical study of human fetal brain evidence was obtained that NA, DA and 5-HT are localized in nerve cell bodies, axons and axon terminals also in the human brain. A detailed mapping of the amine-containing structures was performed and it was found that the general distribution of the monoamine neurons in the human brain—as revealed in the fetuses—were similar to that in the rat brain, though there were differences in details. Of particular interest with respect to function was the microscopical demonstration of a CA-containing nigro-striatal pathway and of a CA-containing tubero-infundibular system in man.

B. In a combined stereotaxic, microspectrofluorometric and fluorescence microscopical study the distribution and amine-content of and the axonal projections from the diencephalic CA-containing nerve cell bodies were analysed. Four main diencephalic cell groups were distinguished: (1) The rostral periventricular DA cell group, (2) the DA cell group of the arcuate nucleus, (3) the dorsal hypothalamic DA cell group, (4) the caudal cell group, comprising both DA-containing and NA-containing cell bodies, extending from the periventricular grey matter of the caudal thalamus down into the posterior hypothalamic area.

The terminal projections from the DA-cells in the arcuate nucleus reach the median eminence-pituitary region. This DA cell group has 3 well-defined projections to the median eminence, the neural lobe and the pars intermedia respectively. In addition, a NA-projection from the lower brain stem to the infundibulum was established. This previously unknown complexity of the median eminence-pituitary CA-innervation is of considerable importance in the functional interpretation of, e.g. pharmacological and endocrine experiments.

The DA-cells in the dorsal hypothalamic cell group and in the caudal cell group seem to constitute a second intradiencephalic DA system, which projects extensively to the subthalamic area, to the zona incerta, and also to the anterior hypothalamic nucleus. This proposed intradiencephalic DA-system, not previously reported, should be considered when dealing with amine functions in the diencephalon.

C. In a fluorescence and electron microscopical study, the fine structural relations between CA-fibres and hormone-producing structures in the rat neuro-intermediate lobe were examined. Evidence was obtained for DA-fibres having close, possibly synaptic, contacts with both neurosecretory fibres and pituicytes in the neural lobe and with endocrine cells in the pars intermedia. These findings indicate a central control of ADH, oxytocin, and MSH production and/or release mediated via DA neurons originating in the arcuate nucleus.

D. In a glyoxylic acid fluorescence study, the adrenergic innervation of rat thalamus was charted. The high precision and specificity of the GA method allowed the demonstration of dense to very dense CA innervations in the different thalamic nuclei. Many areas earlier reported to be largely devoid of CA-fibres were in this investigation found to contain a significant number of fluorescent fibres. It is evident that this prominent adrenergic supply to the thalamus could be of importance, e.g. in motor functions.

E. In a stereotaxic, chemical, and histochemical approach to the organization of indolamine neurons in the rat brain, the neurotoxic dihydroxy-tryptamines, 4,5-DHT, 5,6-DHT, and 5,7-DHT, were used. The mode of action of 5,6-DHT was studied in detail, and evidence was obtained that this drug, within a certain dose range, causes selective degeneration of indolamine neurons, mainly by acting on non-terminal parts of the indolamine axon. 4,5-DHT and 5,7-DHT most probably have the same mode of action.

Both intraventricular and intracerebral injections of 5,6-DHT and 5,7-DHT caused acutely a very pronounced accumulation of yellow-fluorescent material in the indolamine axons, probably by arresting the axo-

plasmic flow and thereby making them visible in the fluorescence microscope. Many important details in the organization of indolamine neurons in the rat brain were revealed with this technique.

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7 REFERENCES

- ANDÉN, N.-E., A. DAHLSTRÖM, K. FUXE, K. LARSSON, L. OLSON and U. UNGERSTEDT, Acta physiol. scand. 1966. 67. 313-326.
- AXELSSON, S., A. BJÖRKLUND, B. FALCK, O. LINDVALL and L.-Å. SVENSSON, Acta physiol. scand. 1973. 87. 57-62.
- AXELSSON, S., A. BJÖRKLUND and O. LINDVALL, J. Histochem. Cytochem. 1972. 20. 435-444.
- AXELSSON, S., A. BJÖRKLUND and N. SEILER, Life Sci. 1971. 10. 745-749.
- BARGMANN, W., E. LINDNER and K.H. ANDRES, Z. Zellforsch. 1967. 77. 282-298.
- BAUMGARTEN, H.G., A. BJÖRKLUND, A.F. HOLSTEIN and A. NOBIN, Z. Zellforsch. 1972. 129. 256-271.
- BAUMGARTEN, H.G., A. BJÖRKLUND, L. LACHENMAYER and A. NOBIN, Acta physiol. scand. 1973 a. Suppl. 391. In press.
- BAUMGARTEN, H.G., A. BJÖRKLUND, L. LACHENMAYER and A. NOBIN, J. Neurochem. 1973b. In press.
- BAUMGARTEN, H.G., A. BJÖRKLUND, L. LACHENMAYER, A. NOBIN and U. STENEVI, Acta physiol. scand. 1971. Suppl. 373. 1-15.
- BAUMGARTEN, H.G., K.D. EVETTS, R.B. HOLMAN, L.L. IVERSEN, M. VOGT and G. WILSON, J. Neurochem. 1972. 19. 1587-1597.
- BAUMGARTEN, H.G. and L. LACHENMAYER, Z. Zellforsch. 1972. 135. 399-414.
- BAUMGARTEN, H.G., L. LACHENMAYER, A. BJÖRKLUND, A. NOBIN and E. ROSENGREN, Life Sci. 1973. In press.
- BAUMGARTEN, H.G., L. LACHENMAYER and H.G. SCHLOSSBERGER, Z. Zellforsch. 1972. 125. 553-569.
- BAUMGARTEN, H.G., J. STEPHEN, J. VICTOR and W. LOVENBERG, J. Neurochem. 1973. In press.

- BELENKY, M.A., M.S. KONSTATINOVA and A.L. POLENOV, Gen. comp. Endocr. 1970. 15. 185-197.
- BERTLER, Å., Acta physiol. scand. 1961. 51. 97-107.
- BERTLER, Å., A. CARLSSON, E. ROSENGREN and B. WALDECK, Kungl. Fysiogr. Sällsk. Lund Förh. 1958. 28. 121-123.
- BERTLER, Å. and E. ROSENGREN, Acta physiol. scand. 1959. 47. 350-361.
- BJÖRKLUND, A., Z. Zellforsch. 1968. 89. 573-589.
- BJÖRKLUND, A., B. EHINGER and B. FALCK, J. Histochem. Cytochem. 1968. 16. 263-270.
- BJÖRKLUND, A., B. EHINGER and B. FALCK, J. Histochem. Cytochem. 1972. 20. 56-64.
- BJÖRKLUND, A., A. ENEMAR and B. FALCK, Z. Zellforsch. 1968. 89. 590-607.
- BJÖRKLUND, A. and B. FALCK, J. Histochem. Cytochem. 1968. 16. 717-720.
- BJÖRKLUND, A. and B. FALCK, Z. Zellforsch. 1969. 93. 254-264.
- BJÖRKLUND, A. and B. FALCK, In: Quantitative Fluorescence Techniques as Applied in Cell Biology (Eds.: A. Thier and M. Sernetz) Springer, Berlin 1973. In press.
- BJÖRKLUND, A., B. FALCK, F. HROMEK, CH. OWMAN and K.A. WEST, Brain Res. 1970. 17. 1-23.
- BJÖRKLUND, A., B. FALCK, O. LINDVALL and L.-Å. SVENSSON, J. Histochem. Cytochem. 1973. 21. 17-25.
- BJÖRKLUND, A., B. FALCK and CH. OWMAN, In: Methods of Investigative and Diagnostic Endocrinology (Ed.: S.A. Berson), Vol. 1: The Thyroid and Biogenic Amines (Eds.: J.E. Rall and I.J. Kopin) North Holland Publ. Comp., Amsterdam 1972. pp. 318-368.
- BJÖRKLUND, A., B. FALCK and E. ROSENGREN, Life Sci. 1967. 6. 2103-2110.
- BJÖRKLUND, A., B. FALCK and U. STENEVI, J. Pharmacol. exp. Ther. 1970. 175. 525-532.
- BJÖRKLUND, A., B. FALCK and U. STENEVI, Brain Res. 1971 a. 32. 269-285.

- BJÖRKLUND, A., B. FALCK and U. STENEVI, In: Progress in Brain Research, Histochemistry of Nervous Transmission (Ed.: O. Eränkö) Elsevier, Amsterdam 1971 b. 34. pp. 63-73.
- BJÖRKLUND, A., R. KATZMAN, U. STENEVI and K.A. WEST, Brain Res. 1971. 31. 21-33.
- BJÖRKLUND, A., O. LINDVALL and L.-Å. SVENSSON, Histochemie 1972. 32. 113-131.
- BJÖRKLUND, A., A. NOBIN and U. STENEVI, Brain Res. 1973. 50. 214-220.
- BJÖRKLUND, A., CH. OWMAN and K.A. WEST, Z. Zellforsch. 1972. 127. 570-579.
- BJÖRKLUND, A. and U. STENEVI, Brain Res. 1971. 31. 1-17.
- BLOOM, F.E., S. ALGERI, A. GROPPETTI, A. REVUELTA and E. COSTA, Science 1969. 166. 1284-1286.
- CANNATA, M.A. and J. H. TRAMEZZANI, Experientia (Basel) 1969. 25. 1281-1282.
- CARLSSON, A., B. FALCK and N.-Å. HILLARP, Acta physiol. scand. 1962. 56. Suppl. 196. 1-28.
- CARPENTER, M.B. and P. PETER, J. Comp. Neurol. 1972. 144. 93-116.
- CORRODI, H. and G. JONSSON, J. Histochem. Cytochem. 1967. 15. 65-78.
- DAHLSTRÖM, A. and K. FUXE, Acta physiol. scand. 1964. 62. Suppl. 232. 1-55.
- DAHLSTRÖM, A. and K. FUXE, Acta physiol. scand. 1965. 64. Suppl. 247. 1-36.
- DAHLSTRÖM, A. and K. FUXE, Acta Endocr. 1966. 51. 301-314.
- DALY, J., K. FUXE and G. JONSSON, Brain Res. 1973. 49. 476-482.
- DE LA TORRE, J.C., Acta neuropath. (Berlin) 1972. 21. 165-168.
- EHINGER, B., Z. Zellforsch. 1966. 71. 146-152.
- EHRINGER, H. and O. HORNYKIEWICZ, Wien. klin. Wschr. 1960. 38. 1236-1239.
- FAHN, S., L.R. LIBSCH and R.W. CUTLER, J. neurol. Sci. 1971. 14. 427-455.

- FALCK, B., Acta physiol. scand. 1962. 56. Suppl. 197. 1-25.
- FALCK, B., N.-Å. HILLARP, G. THIEME and A. TORP, J. Histochem. Cytochem. 1962. 10. 348-354.
- FALCK, B. and CH. OWMAN, Acta Univ. Lund II 1965. 7. 1-23.
- FLEMING, R.M. and W.G. CLARK, J. Chromatogr. 1970. 52. 305-312.
- FUXE, K., Z. Zellforsch. 1964. 61. 710-724.
- FUXE, K., Acta physiol. scand. 1965. 64. Suppl. 247. 37-85.
- FUXE, K., B. HAMBERGER and T. MALMFORS, J. Pharm. Pharmacol. 1966. 18. 543-544.
- FUXE, K. and T. HÖKFELT, Acta physiol. scand. 1966. 66. 243-244.
- FUXE, K., T. HÖKFELT and U. UNGERSTEDT, Advanc. Pharmacol. 1968. 6. 235-251.
- FUXE, K., T. HÖKFELT and U. UNGERSTEDT, In: Metabolism of Amines in the Brain (Ed.: E. Hooper) Mac Millan, London 1969. pp. 10-22.
- GREEN, A.R., S.H. KOSLOW and E. COSTA, Brain Res. 1973. 51. 371-374.
- HAMBERGER, B., Acta physiol. scand. 1967. Suppl. 295. 1-47.
- HALÁSZ, B. and L. PUPP, Endocrinology 1965. 77. 553-562.
- HELLER, A., J.A. HARVEY and R.Y. MOORE, Biochem. Pharmacol. 1962. 11. 859-866.
- HORNYKIEWICZ, O., Pharmacol. Rev. 1966. 18. 925-964.
- HÄGGENDAL, J., Acta physiol. scand. 1963. 59. 242-254.
- HÄGGENDAL, J. and T. MALMFORS, Acta physiol. scand. 1965. 59. 295-296.
- HÖKFELT, T., Z. Zellforsch. 1968. 91. 1-74.
- HÖKFELT, T. and K. FUXE, In: Brain-Endocrine Interaction (Eds.: K.M. Knigge, D.E. Scott and A. Weindl) Karger, Basel 1972. pp. 181-223.
- HÖKFELT, T. and Å. LJUNGDAHL, Histochemie 1972. 29. 325-339.
- JONSSON, G., K. FUXE and T. HÖKFELT, Brain Res. 1972. 40. 271-281.
- JOYCE, D., Brit. J. Pharmacol. 1962. 18. 370-380.
- KATZMAN, R., A. BJÖRKLUND, CH. OWMAN, U. STENEVI and K.A. WEST, Brain Res. 1971. 25. 579-596.

- KUHAR, M.J., G.K. AGHAJANIAN and R.H. ROTH, Brain Res. 1972. 44. 165-176.
- KUHAR, M.J., R.H. ROTH and G.K. AGHAJANIAN, Brain Res. 1971. 35. 167-176.
- KÖNIG, J.F.R. and R.A. KLIPPEL, The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Williams and Wilkins, Baltimore 1963.
- LIDBRINK, P., G. JONSSON and K. FUXE, Neuropharmacology 1971. 10. 521-536.
- LICHTENSTEIGER, W. and H. LANGEMANN, J. Pharmacol. exp. Ther. 1966. 151. 400-408.
- LINDVALL, O. and A. BJÖRKLUND, Histochemie 1973. In press.
- LINDVALL, O., A. BJÖRKLUND, T. HÖKFELT and Å. LJUNGDAHL, Histochemie 1973. In press.
- LUFT, J.H., J. biophys. biochem. Cytol. 1961. 9. 409-414.
- MAEDA, T. and N. SHIMUZU, Brain Res. 1972. 36. 19-31.
- MALMFORS, T., Acta physiol. scand. 1963. 58. 99-100.
- MALMFORS, T. and CH. SACHS, Europ. J. Pharmacol. 1968. 3. 89-92.
- McCANN, S.M., P.S. KALRA, A.O. DONOSO, W. BISHOP, H.P. SCHNEIDER, C.P. FAWCETT and L. KRULICH, In: Brain-Endocrine Interaction (Eds.: K.M. Knigge, D.E. Scott and A. Weindl) Karger, Basel 1972. pp. 224-235.
- McGEER, E.G. and P.L. McGEER, Canad. J. Biochem. Physiol. 1962. 40. 1141-1151.
- McILWAIN, H. and R. RODNIGHT, Practical neurochemistry, J. and A. Churchill, London 1962.
- MOORE, R.Y., Int. Rev. Neurobiol. 1970. 13. 67-91.
- MOORE, R.Y., R.K. BHATNAGAR and A. HELLER, Brain Res. 1971. 30. 119-135.
- MOORE, R.Y., A. BJÖRKLUND and U. STENEVI, Brain Res. 1971. 33. 13-35.

- MOORE, R.Y. and A. HELLER, J. Pharm. exp. Ther. 1967. 156. 12-22.
- OLSON, L. and K. FUXE, Brain Res. 1972. 43. 289-295.
- POIRIER, L.J., P. LANGELIER, A. ROBERGE, R. BOUCHER and A. KITSIKIS, J. Neurol. Sci. 1972. 16. 401-416.
- REYNOLDS, E.S., J. Cell Biol. 1963. 17. 210-213.
- RITZEN, M., M.D. Thesis, P.A. Norstedt, Stockholm 1967.
- SACHS, CH., Acta physiol. scand. 1970. Suppl. 341. 1-50.
- SACHS, CH. and G. JONSSON, J. Neurochem. 1972. 19. 1561-1575.
- SANO, I., T. GAMO, Y. KAKIMOTO, K. TANIGUCHI, M. TAKESADA and K. NISHINUMA, Biochim. Biophys. Acta (Amst.) 1959. 32. 586-587.
- SHASKAN, E.G. and S.H. SNYDER, J. Pharmacol. exp. Ther. 1970. 175. 404-418.
- THOENEN, H. and J.P. TRANZER, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. 1968. 261. 271-288.
- TRANZER, J.P. and H. THOENEN, Experientia (Basel) 1967. 23. 743-745.
- TRANZER, J.P. and H. THOENEN, Experientia (Basel) 1968. 24. 155-156.
- UNGERSTEDT, U., Europ. J. Pharmacol. 1968. 5. 107-110.
- UNGERSTEDT, U., Acta physiol. scand. 1971 a. 367. 1-48.
- UNGERSTEDT, U., In: 6-Hydroxydopamine and Catecholamine Neurons (Eds.: T. Malmfors and H. Thoenen) North Holland Publ., Amsterdam 1971 b. pp. 101-127.
- URETSKY, N.J. and L.L. IVERSEN, J. Neurochem. 1970. 17. 269-278.
- WEMAN, B. and A. NOBIN, Z. Zellforsch. 1973. In press.
- VINCENT, D.S. and T.C. ANAND KUMAR, Z. Zellforsch. 1969. 99. 185-197.

